E. coli by Membrane Filtration using Modified mTEC Agar
EPA 1603 – December 2009

Facility Name:____VELAP ID___________________

Assessor Name:_________________Analyst Name:_______________Inspection Date_______________

Relevant Aspect of Standards	Method Reference	Y	N	N/A	Comments
Records Examined: SOP Number/ Revision/ Date _________________________ Analyst:_______________					
Sample ID: ________________ Date of Sample Preparation:___________ Date of Analysis:_____________					
1) Are samples transported at <10°C?	8.1.2				
2) Is the maximum sample holding time 8 hours?	40CFR136.3(e) Table II				
3) Are pipets and membrane filter units sterilized and kept wrapped in foil or kraft paper?	6.6				
4) Are membrane filters sterile, white, grid marked and 47mm diameter, with 0.45±0.02µm pore size?	6.20				
5) Is sample incubator maintained at 35°C±0.5°C, with approximately 90% humidity if loose-lidded petri dishes are used?	6.23				
6) Is the water bath for sample incubation maintained at 44.5±0.2˚C?	6.24				
7) Is the water bath for tempering agar maintained at 50˚C?	6.25				
8) Is stock phosphate buffer solution prepared as follows? KH_2PO_4…………………………………..34.0 g Reagent grade water…………………….…1.0 L	7.5.1				
9) Is stock magnesium chloride solution prepared as follows? Anhydrous $MgCl_2$……………….………..38.0 g Reagent grade water……………………1.0 L OR $MgCl_2 \cdot 6H_2O$ ($MgCl_2$ hexahydrate)…...81.1 g Reagent grade water……….……………1.0 L	7.5.2				

<u>*Notes/Comments*</u>

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10) Is working phosphate buffered dilution water/rinse water prepared as follows? Mix 1.25 mL of the stock phosphate buffer and 5 mL of the $MgCl_2$ stock per liter of reagent-grade water. Is final pH 7.0±0.2?	7.5.4				
11) Are stock and working phosphate buffer solutions sterilized by autoclaving at 121˚C for 15min? Are the stock solutions stored in the refrigerator?	7.5.1 7.5.3				
12) Is the modified mTEC Agar prepared by adding 45.6g dehydrated powder to 1 L reagent-grade water, heated until dissolved, autoclaved at 121°C for 15 min, and cooled in a 50°C water bath? [NOTE: Check composition of agar against specifications in 7.6.1]	7.6.1 7.6.2				
13) Is the modified mTEC agar final pH 7.3 ± 0.2?	7.6.2				
14) Is mTEC agar dispensed into 9 × 50 mm or 15 × 60 mm petri dishes to a 4-5 mm depth (approximately 4-6 mL), and allow to solidify?	7.6.2				
15) Is each petri dish and report form marked with the sample identification and volume?	11.2				
16) Is an initial filtration blank prepared by filtering 50-mL of phosphate-buffered dilution water and placing the filter on a Tryptic Soy Agar plate, incubating for 24 ± 2 hours at 35°C ± 0.5°C and checking for growth?	9.10				
17) Is the sample shaken vigorously at least 25 times?	11.4				
18) Are sample volumes chosen to produce 20-80 *E. coli* colonies? (Multiple volumes of the same sample or sample dilutions may be filtered and the results combined.)	11.5 11.6				

<u>*Notes/Comments*</u>

METHOD CHECKLISTS ARE AN INTERVIEW TOOL USED BY ASSESSORS AND ARE NOT TO BE CONSIDERED AS A SUBSTITUTE FOR REQUIREMENTS OF THE PUBLISHED METHOD. CHECKLISTS ARE SUBJECT TO CHANGE.

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19) Is a sterile membrane filter placed on the filter base grid side up with sterile smooth-tipped forceps, and funnel attached to the base with membrane filter held between the funnel and the base?	6.13 11.3				
20) Are smaller sample sizes or sample dilutions used to minimize the interference of turbidity or for high bacterial densities? When analyzing smaller sample volumes (<20 mL),are 20-30 mL of phosphate buffered water added to the funnel or is an aliquot of sample dispensed into a dilution blank prior to filtration?	11.6				
21) After the sample is filtered, are the sides of the funnel rinsed at least twice with 20-30mL portions of sterile buffered rinse water?	11.7				
22) Is the filter aseptically removed and rolled onto the modified mTEC agar to avoid the formation of bubbles?	11.8				
23) Is the sample dish inverted, incubated at 35±0.5˚C for 2±0.5 hrs, transferred to a Whirl-pak® bag, sealed, inverted and incubated in a water bath at 44.5±0.2˚C for 22±2 hrs?	11.8 11.9				
24) After 22±2 hrs, are plates removed from water bath and red or magenta colonies counted under fluorescent light with 2 to 5 X magnification and recorded?	6.2 11.10				
25) Is the number of colonies calculated using this formula? $\text{E.coli / 100 mL} = \frac{\text{colonies counted}}{\text{mL sample filtered}} \times 100$	13.1				
26) Are results reported as _E. coli_ CFU/100 mL?	13.3				
27) Are multiple plates counted and calculated according to App. B?	App. B				

Notes/Comments

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28) **Ongoing precision and recovery (OPR) –** Does the laboratory routinely process and analyze spiked phosphate buffered saline (PBS) samples to demonstrate ongoing control of the analytical system? Does the laboratory analyze one OPR sample after every 20 field and matrix spike samples or one per week that samples are analyzed, whichever occurs more frequently? OPR samples must be accompanied by an acceptable method blank (§9.9) and appropriate media sterility checks (§9.11). Are the OPR analysis is performed as described in §9.4.1 – 9.4.4?	9.4				
29) Does phosphate buffered saline (PBS) have the following composition? Monosodium phosphate (NaH_2PO_4) 0.58 g Disodium phosphate (Na_2HPO_4) 2.5 g Sodium chloride 8.5 g Reagent-grade water 1.0 L	7.4.1				
30) Are the ingredients dissolved in 1 L of reagent-grade water, and dispensed in appropriate amounts for dilutions in screw cap bottles or culture tubes, and/or into containers for use as rinse water? Is PBS autoclaved at 121°C (15 PSI) for 15 minutes? Is final pH 7.4 ± 0.2?	7.4.2				
31) Are spiking suspensions prepared in the laboratory as described in §14.2 or are BioBalls used as described in §14.3?	14.2 14.3				
32) Spike a 100-mL PBS sample with *E. coli* ATCC #11775 according to the spiking procedure in §14. Filter and process each OPR sample according to the procedures in §11 and calculate the number of *E. coli* per 100 mL according to §13.	9.4.1				
33) Calculate the percent recovery (R) for the OPR sample using the appropriate equation in §14.2.4.3 for samples spiked with laboratory-prepared spiking suspensions or §14.3.2 for BioBalls, respectively.	9.4.2				

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34) Compare the OPR result (percent recovery) with the corresponding OPR recovery criteria in Table 1. If the OPR result meets the acceptance criteria for recovery, method performance is acceptable and analysis of field samples may continue. If the OPR result falls outside of the acceptance criteria, system performance is unacceptable. In this event, identify the problem by evaluating each step of the analytical process (media, reagents, and controls), correct the problem and repeat the OPR analysis.	9.4.3				
35) Ongoing Precision and Recovery (OPR) Acceptance Criteria <u>Performance test</u> / <u>Lab-prepared spike acceptance criteria</u> / <u>BioBall™ acceptance criteria</u> OPR as percent recovery / 38% - 127% / detect - 144%	Table 1.				
36) As part of the laboratory QA program, are results for OPR samples charted and updated records maintained in order to monitor ongoing method performance? The laboratory should also develop a statement of accuracy for Method 1603 by calculating the average percent recovery (R) and the standard deviation of the percent recovery (s_r). Express the accuracy as a recovery interval from R - 2 s_r to R + 2s_r.	9.4.4				

<u>*Notes/Comments*</u>

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Relevant Aspect of Standards	Method Reference	Y	N	N/A	Comments
37) **Matrix spikes (MS) -** MS analysis are performed to determine the effect of a particular matrix on *E. coli* recoveries. Does the laboratory analyze one MS sample when disinfected wastewater samples are first received from a source from which the laboratory has not previously analyzed samples? Subsequently, do 5% of field samples (1 per 20) from a given disinfected wastewater source include a MS sample? MS samples must be accompanied by the analysis of an unspiked field sample sequentially collected from the same sampling site, an acceptable method blank (§9.9), and appropriate media sterility checks (§ 9.11). When possible, MS analyses are accompanied by an OPR sample (§ 9.4), using the same spiking procedure (laboratory-prepared spiking suspension or BioBalls). The MS analysis is performed as follows:	9.5				
38) Prepare two, 100-mL field samples that were sequentially collected from the same site. One sample will remain unspiked and will be analyzed to determine the background or ambient concentration of *E. coli* for calculating MS recoveries (§9.5.3). The other sample will serve as the MS sample and will be spiked with *E. coli* ATCC #11775 according to the spiking procedure in §14.	9.5.1				
39) Select sample volumes based on previous analytical results or anticipated levels of *E. coli* in the field sample in order to achieve the recommended target range of *E. coli* (20-80 CFU, including spike) per filter. If the laboratory is not familiar with the matrix being analyzed, analyze a minimum of three dilutions to ensure that a countable plate is obtained for the MS and associated unspiked sample. Analyze 100-mL of sample.	9.5.2				

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40) Spike the MS sample volume(s) with a laboratory-prepared suspension as described in §14.2 or with BioBalls as described in §14.3. Immediately filter and process the unspiked and spiked field samples according to the procedures in §11. *Note:* When analyzing smaller sample volumes (e.g, <20 mL), 20-30 mL of PBS should be added to the funnel or an aliquot of sample should be dispensed into a 20-30 mL dilution blank prior to filtration. This will allow even distribution of the sample on the membrane.	9.5.3				
41) For the MS sample, calculate the number of *E. coli* (CFU/100 mL) according to §13 and adjust the colony counts based on any background *E. coli* observed in the unspiked matrix sample.	9.5.4				
42) Calculate the percent recovery (R) for the MS sample (adjusted based on ambient *E. coli* in the unspiked sample) using the appropriate equation in §14.2.4.3 or 14.3.2 for samples spiked with laboratory-prepared spiking suspensions or BioBalls, respectively.	9.5.5				
43) $R = 100 \times \frac{(N_s - N_u)}{T}$	14.2.4.3 14.3.2				
44) Compare the MS result (percent recovery) with the appropriate method performance criteria in Table 2. If the MS recovery meets the acceptance criteria, system performance is acceptable and analysis of field samples from this disinfected wastewater source may continue. If the MS recovery is unacceptable and the OPR sample result associated with this batch of samples is acceptable, a matrix interference may be causing the poor results. If the MS recovery is unacceptable, all associated field data should be flagged.	9.5.6				

Notes/Comments

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Relevant Aspect of Standards	**Method Reference**	**Y**	**N**	**N/A**	**Comments**
45) Acceptance criteria for MS recovery (Table 2) are based on data from spiked disinfected wastewater matrices and are not appropriate for use with other matrices (e.g., ambient recreational waters).	9.5.7				
46) Matrix Spike Precision and Recovery Acceptance Criteria <u>Performance test</u> — <u>Lab-prepared acceptance criteria</u> — <u>BioBall™ acceptance criteria</u> Percent Recovery for MS — 12% - 149% — 17% - 117%	Table 2				
47) Record and maintain a control chart comparing MS recoveries for all matrices to batch-specific and cumulative OPR sample results analyzed using Method 1603. These comparisons help laboratories recognize matrix effects on method recovery and recognize inconsistent or sporadic matrix effects from a particular source.	9.5.8				

<u>Notes/Comments</u>